Detection of HSV in CSF using the Focus Diagnostics Simplexa™ HSV 1 & 2 Direct Sample-to-Answer Real-time PCR Assay

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Background: Rapid detection of Herpes Simplex Virus (HSV) infections of the central nervous system with PCR is standard-of-care for patient management, especially in pediatric institutions. However, there are no commercially available HSV assays FDA-cleared for use in cerebrospinal fluid (CSF). HSV is one of the leading causes of aseptic meningitis with HSV-2 being the 2nd most common etiology after enterovirus. While HSV meningitis is often self-limited, when the infection includes encephalitis or meningoencephalitis, the mortality rate of untreated cases is nearly 50%. An FDA-cleared assay that is simple to use could significantly impact patient care.

The goal of this study was to compare the performance of the Simplexa™ HSV 1 & 2 Direct (Focus Diagnostics) real-time PCR assay (Simplexa) with a lab-developed real-time PCR assay (LDA) for the detection of HSV in CSF. The Simplexa assay is performed on the 3M Integrated Cycler and recently received FDA-clearance.

Methods: Seventy-nine archived pediatric and adult CSF specimens previously characterized with the LDA were tested using Simplexa. The Simplexa assay targeted the HSV polymerase gene using 50µL of CSF loaded directly into the disk without prior extraction. The assay targets the HSV glycoprotein B gene using the LightCycler (Roche). Discordant specimens were re-extracted and retested in the LDA only. Truth was determined based on clinical correlation. Relative analytical sensitivities and limits of detection (LoD) studies were performed using AcroMetrix® HSV-1 & 2 Control Panels (Life Technologies Inc.).

Results: Of 79 CSF specimens tested, the Simplexa assay and LDA results were in agreement in 71/75 (94.7%). Simplexa detected 47/51 HSV-positive CSF samples accurately and did not detect HSV in 24/24 HSV-negative samples for a relative sensitivity of 92.2% specificity of 100%. The analytical LoD of the Simplexa was between 2.5 copies/reaction for both HSV-1 & HSV-2. Seventy-nine archived pediatric and adult CSF specimens previously characterized with the LDA were tested using Simplexa.

Patient Samples: Seventy-nine archived pediatric and adult CSF specimens previously characterized with the LDA were tested using Simplexa

Lab Developed Assay: For the LDA we extract 400µL of CSF and elute into 55µL using a bioMerieux easyMag. The PCR uses 5µL of the eluate and targets the HSV glycoprotein B gene. HSV-1 and HSV-2 are detected by differential melting peaks using a Roche LightCycler.

Simplexa HSV 1 & 2 Direct
Is a real-time PCR assay for the detection and differentiation of HSV-1 & -2 DNA in CSF samples. Extraction and amplification is performed in one protocol without prior nucleic acid extraction. The assay targets the HSV polymerase gene using 50µL of CSF loaded directly into the direct amplification discs. Each disc has capacity for up to 8 reactions.

Discordant Specimens: Discordant specimens were re-extracted and retested in the LDA only due to limited sample volume. Truth was determined based consensus after repeat testing.

Analytical Comparison:
Relative analytical sensitivity and limits of detection (LoD) studies were performed using HSV-1 & 2 Control Panels from AcroMetrix® (Life Technologies Inc.) and Zepptomx.

Analytical Comparison:
Relative analytical sensitivity and limits of detection were similar for both the LDA and the Simplexa assay (data not shown). For HSV-1 the limit of detection was 2 copies/rxn (≈25-50 c/mL) and for HSV-2 it was 3-5 c/rxn (≈100-150 c/mL).

Discussion:
During the method comparison we observed 4 discordant results and upon repeat analysis with the LDA the results were confirmed. Due to limitations in the volume of the archived samples we could not repeat both assays.

• The Cts of the for discordant results were all ≥ 34 with 2 results > 37 in the LDA. These latter 2 results are near the 95% detection limits of the Simplexa assay.

• There was a single discordant typing which LDA typed as HSV-1 and Simplexa typed as HSV-2. The patient also had blood, genital and skin lesions positive for HSV-1 by the LDA. Sequencing by Focus for two targets indicates the isolate is HSV-2; additional testing is underway.

• Analytically, the Simplexa assay appears to have limits of detection similar to that of the LDA, however we were unable to test enough samples at the LOD to derive definitive limits.

• The Focus Diagnostics Simplexa HSV 1 & 2 Direct Molecular Test received FDA Clearance. The product achieved moderate complexity status for use in CLIA moderate and high complexity healthcare facilities.

• Due to its ease of use, time to result and comparable performance to our well defined LDA, the Simplexa HSV-1&2 Direct test would be a reasonable addition to the molecular test menu especially for laboratories that currently send samples to a reference lab for HSV testing.

Acknowledgement:
We thank Focus Diagnostics for supplying the Simplexa HSV 1 & 2 Direct Molecular Test kits (UO marked) used for this evaluation.

Table 1. Patient CSF Results

<table>
<thead>
<tr>
<th></th>
<th>Simplexa +</th>
<th>Simplexa neg</th>
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</thead>
<tbody>
<tr>
<td>LDA +</td>
<td>47</td>
<td>4</td>
</tr>
<tr>
<td>LDA neg</td>
<td>0</td>
<td>28</td>
</tr>
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Table 2. Distribution of HSV Types

<table>
<thead>
<tr>
<th></th>
<th>HSV-1</th>
<th>HSV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplexa</td>
<td>3</td>
<td>44</td>
</tr>
<tr>
<td>LDA</td>
<td>4</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 2. Distribution of HSV Types:
Of the 47 samples positive by both Simplexa and the LDA, there was a single sample with discordant typing (Simplexa negative /LDA positive).